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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
FORD, ALLISON M	
ART UNIT	PAPER NUMBER
1651	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/505,252	ROMAGNE ET AL.	
	Examiner	Art Unit	
	ALLISON M. FORD	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *31 October 2008*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *21,23-25,27-34 and 51-54* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *21,23-25,27-34 and 51-54* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicants' response of 10/31/2008 has been received and entered into the application file. Claim 21 has been amended; claims 1-20, 22, 26 and 35-50 have been cancelled; new claims 53-54 have been added. Claims 21, 23-25, 27-34 and 51-54 remain pending in the current application, all of which have been considered insofar as they read on the elected species of "phosphohalohydrins" as the selective activator of gamma delta T lymphocytes, claim 31 limited to BrHPP.

Response to Arguments/Amendments

Applicant's remarks of 10/31/2008 have been received and fully considered. The declaration by S. Salot dated 10/31/2008 has also been received and fully considered. Each of the grounds of traversal will be addressed below, as appropriate. Objections/Rejections not repeated herein are withdrawn.

Priority

Acknowledgment is made of the instant application being a 371 application of international application PCT/FR03/000585 on 2/21/2003. Acknowledgement is further made of the claim for foreign priority under 35 USC 119(a)-(d) to application FR 02/02305, filed 2/22/2002; a certified copy of the foreign priority documents have been received in the instant application.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Applicants have removed the term 'biological preparation' from claim 21 in an effort to expedite prosecution; however the rejection stands, as it was not the actual term 'biological preparation' that was indefinite, but rather whether the sample (whether called a biological preparation or just a blood or cytapheresis sample) initially contains gamma delta T lymphocytes or not.

Claims 21, 23-25, 27-34 and 51-54 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In claim 21, it is not clear if the blood sample or cytapheresis sample initially contains gamma delta T lymphocytes, and the method is intended to enrich the proportion of gamma delta T lymphocytes within the sample; or if the biological preparation may contain any mononuclear cells, and the method is intended to involve transdifferentiation of various (non-gamma delta T lymphocytes) mononuclear cells into gamma delta T lymphocytes. As is, the claims do not currently make the intent of the method clear, or the steps necessary to achieve the method, if a transdifferentiation step is required such must be expressly claimed. Clarification is required.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Applicants' arguments received with the response of 10/31/2008 have been fully considered, but are not found persuasive to overcome the rejection of record.

Applicants have traversed the rejections of record on the basis that the showings of Skea et al, with regards to gamma delta T lymphocyte proliferation over 27 days, may not be extrapolated to support a conclusion that the duration of culture period directly effects the proportion of gamma delta T lymphocytes. This argument is based on the fact that the cited example of Skea et al used only 2 ng/mL of IL-2 (equivalent to only about 33 U/mL of IL-2), which is well below the level required by new claims 53 and 54. Furthermore, Applicants assert that the concentration of IL-2 used by Belmant et al and/or Espinosa et al may not be considered a result effective variable which would have been routinely optimized, because the required range is significantly outside the range disclosed by either reference. Applicants have cited *In re Sebek* in support.

These arguments are found persuasive, the data provided by Skea et al was generated using significantly different culture conditions that those of Belmant et al or Espinosa et al, and thus the effect of the concentrations of the cytokines and the duration of the culture period may not be extrapolated to the methods of Belmant et al or Espinosa et al. However, new grounds of rejection have been set forth below, relying on the teachings of Garcia et al with respect to the concentration of cytokines. Please note the only claims that specifically require the IL-2 to be present in a concentration of 150-500 U/mL are new claims 53 and 54, therefore, the new grounds of rejections are necessitated by the addition of the new claims.

Regarding the declaration by Mr. Salot:

At point 3, Mr. Salot addresses the accuracy of the statements in the rejection of record regarding optimization of cell culture density as a result effective variable. Mr. Salot asserts that cell concentrations of greater than 5×10^6 cells/mL (5 million cells/mL), such as that employed by Belmont et al or Espinosa et al, would not be employed because such high concentrations would not favor survival of the cells.

In response, it is respectfully submitted that neither Belmont et al nor Espinosa et al disclose a cell concentration of 5 x 10^6 cells/mL or greater, rather both references disclose providing an initial cell concentration of 1 x 10^6 cells/mL (1 million cells/mL), which is within the claimed ranges. Furthermore, Skea et al was further cited for their disclosure of maintaining gamma delta T cell cultures at even lower concentrations of 1 x 10^5 cells/mL. Thus, the references' cell concentrations were well below 5 x 10^6 cells/mL, and well within the claimed range.

At point 4, Mr. Salot addresses the differences between the method of Skea et al and those of Belmant et al, specifically highlighting the differences in cytokine levels. Mr. Salot attests that one skilled in the art would not reasonable apply the conclusions found by Skea et al to be applicable to the methods of Belmant et al.

In response, this point has been well taken, and the rejection of record has been amended to reflect such an understanding.

Claims 21, 23-25, 27-34 and 51-54 are rejected under 35 U.S.C. 103(a) as being unpatentable over Belmant et al (US Patent 6,660,723), in view of Skea et al (Journal of Hematotherapy and Stem Cell Research, Oct 2001), Garcia et al (Journal of Immunology, 1998) and Valeri (Blood Banking and the Use of Frozen Blood Products, 1976).

Belmant et al teach a method for enriching the concentration of gamma delta T lymphocytes (Tγ9δ2 lymphocytes) in a cell sample (which reads on what Applicants are calling preparing a gamma delta T lymphocyte composition).

Example 10 of Belmant et al is relied upon: Belmant culture 1 mL of a blood extract comprising 10^6 T lymphocytes in culture medium comprising inactivated human serum, 0.1-100 nM BrHPP or IHPP, and 50 U/mL of IL-2. After 4 days Belmant et al report adding another 50

U/mL of IL-2 to the culture, thereby increasing the IL-2 concentration to 100 U/mL (See Belmant et al, col. 2, ln 44-65 & specifically Example 10, col. 22, ln 1-34). The proportion of delta gamma T lymphocytes was reported as increasing from 1-5%, in the initial sample, to 10% to greater than 50% in the presence of BrHPP and to 10% to about 80% in the presence of IHPP (See Fig. 1).

Belmant et al differ from the instant invention in that they do not disclose the same specific culture parameters recited in the instant claims, including the original cell count, cell density during culture, culture duration, or concentration of the cytokine. However, it is maintained that it would have been well within the purview of one of ordinary skill in the art to optimize the duration of the culture period, the initial cell number, the culture density during culture, and the concentration of the IL-2 to those values recited in the instant claims, as each of the parameters was recognized in the art as acceptable values for T lymphocyte cell culture, and/or were recognized as result effective variables, which would have been routinely optimized by the artisan of ordinary skill.

With regards to the cell count of the initial cell sample, the current claims require an initial sample comprising at least 50 million mononuclear cells. The sample of Belmant et al only comprises 1 million mononuclear cells (10^6 T lymphocytes, see Belmant et al, col. 22, ln 8). However it is maintained that, in the field of cell culture, the starting cell count is generally recognized to be a result effective variables that directly affect the final cell number produced by the culture. Therefore, the culture of Belmant et al may be 'scaled up' to involve 50-100 mL of sample, which would contain 50-100 million cells. The scaling up of the original sample size would produce a correspondingly greater number of delta gamma T lymphocytes in the end, including over 100 million functional and viable gamma delta T cells.

With regards to the concentration maintained throughout the culture period, Belmant et al reports providing the cells at a concentration of 1 x 10^6 cells/mL (See Belmant et al, col. 22, ln 8-9), which is less than 5 x 10^6 cells/mL. Presumably Belmant et al maintain the cell culture at this density, though they do not explicitly state such. However, Skea et al is relied upon to show the art taught cultures of mononuclear cells for enrichment of gamma delta T lymphocytes were routinely maintained at densities of 1 x 10^5 cells/mL (See Skea et al, Pg. 526, col. 1, "T Cell Culture"). Therefore, it is submitted that one of ordinary skill in the art, at the time the invention was made, would have been able to determine an appropriate concentration at which to maintain the cell culture of Belmant et al, the concentration being less than 5 x 10^6 cells/mL. (claims 21, 24, 29-32, 34 and 51)

With regards to the duration of culture, while Example 10 involves culturing the cells for 8 days (which, itself, may be considered to read on "about 10 days" (claim 27)), it is noted that Example 14 reports in vitro culturing of lymphocytes for a period of 10 days (See Belmant et al, col. 25, Table II). Therefore, the lymphocytes may be maintained in culture for up to at least 10 days, and presumably longer, as there is no requirement that the culture continues to increase in proportion of gamma delta T lymphocytes over time; once the culture has reached its maximum percentage of gamma delta T lymphocytes, it would be reasonably expected that the culture may simply be maintained. (claims 27 and 28)

With regards to the concentration of the IL-2 provided in the culture, while Belmant et al disclose using 50-100 U/mL of IL-2, the concentration of the cytokines was recognized as a result effective variable that would have been routinely optimized by the artisan of ordinary skill. In support, see Garcia et al. Garcia et al measure gamma delta T cell response to IL-2, and report that in three out of four cell clones tested, the proliferation rate of gamma delta T cells increased

with increasing concentrations of IL-2 (See Garcia et al, Fig. 2). In all four cell clones tested, an increase was achieved with 10 ng/mL of IL-2 (10 ng/mL = approximately 163 U/mL, using conversion factor of 1 ng = 16.3 U). Therefore, use of IL-2 at a concentration of 163 U/mL was recognized as a concentration at which gamma delta T lymphocytes are successfully activated, and thus optimization to such a range would have been within the purview of one of ordinary skill in the art, as the general range was taught by the art (i.e. Garcia et al). (claims 33, 53, 54)

Generally, differences in concentration will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration is critical or produces unexpected results. Where the general conditions of a claim are disclosed by the prior art it is not inventive to discover the optimum or workable ranges by routine experimentation, See *In re Aller,* 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

Still further, regarding the cytokines used in the experiments of Belmant et al, while Belmant et al added IL-2 to their media, it would further have been obvious to one of ordinary skill in the art at the time the invention was made to alternatively add IL-15 to the culture medium, as recited in claims 21 and 52. In support see Garcia et al; Garcia et al teach that specific cytokines, including IL-2 and IL-15 are known to enhance gamma delta T lymphocyte activation in the presence of an activator of gamma delta T cells (See Garcia et al, Pg. 4324 & Fig. 2). Garcia et al unexpectedly found IL-15 to be a more potent induces of gamma delta T cell proliferation than IL-2 (See Garcia et al, Pg. 4324, col. 2). Therefore, it would have been well within the purview of one of ordinary skill in the art to alternatively utilize IL-15 in place of, or in addition to, the IL-2 used by Belmant et al (Claims 21 & 32). One of ordinary skill in the art would be motivated to substitute IL-15 for IL-2 in the method of Belmant et al because Garcia et al teach IL-15 is more potent than IL-2 in enhancing gamma delta T cell proliferation. One would expect success based on the experimental findings of Garcia et al, which show successful

activation and proliferation of gamma delta T cells in the presence of IL-15 and an activator compound. Regarding the concentration of IL-15 used, Garcia et al use of up to 100 ng/mL of IL-15, but report the effect of IL-15 on gamma delta T cell proliferation was dose dependent (See Garcia et al, Pg. 4324, col. 2). Therefore, while applicant specifically claims a cytokine concentration in the range of 150 U/mL to 500 U/mL, manipulation and optimization within this range would be well within the purview of one of ordinary skill in the art, again based on the desired activation rate of the cells as well as how often the cells are passaged (Claims 33 and 52).

Finally, while Belmant et al teaches the gamma delta T lymphocytes can be from a blood sample or blood extract, they do not specifically teach separating whole blood by cytapheresis prior to the culture method described above, nor do they teach using previous frozen samples of blood. However, at the time the invention was made it would have been well within the purview of one of ordinary skill in the art to obtain a blood sample at a first point in time, separate the whole blood sample by cytapheresis into individual components, freeze the individual components by appropriate means known in the art, and then at later time, thaw the desired platelet component for use in the method of Belmant et al (Claims 23 and 25). In support see Valeri; Valeri teaches the basic protocols for obtaining, separating and storing blood. Valeri teaches cytapheresis allows separation of specific components of blood from whole blood (See Valeri, Pg. 1, col. 1). Separating the platelets via 'plateletpheresis' removes the majority of red blood cells and plasma components, thereby increasing the proportion of gamma delta T lymphocytes present in the initial cell sample, which is desirable in the method of Belmant et al, as it would ensure a higher proportion of gamma delta T lymphocytes for culture and expansion, resulting in a greater end cell count. Additionally, Valeri teaches methods for frozen storage of separated platelets (See Valeri, Pg 297), which is desirable when the blood sample must be stored for a period of greater than 4 hours prior to use in the method of Belmant et al (See Valeri, Pg. 6,

col. 1). One would expect success separating the desired platelet component via cytapheresis and storing the separated components in a frozen state for future use, because such methods and procedures are common in the art of hematology, as demonstrated by the teachings of Valeri. (claims 23 and 25)

Therefore the invention as a whole would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Claims 21, 23-25, 27-34 and 51-54 are rejected under 35 U.S.C. 103(a) as being unpatentable over Espinosa et al (Journal of Biological Chemistry, 2001), in view of Skea et al (J Hematotherapy & Stem Cell Res, 2001), Garcia et al (Journal of Immunology, 1998) and Valeri (Blood Banking and the Use of Frozen Blood Products, 1976).

Espinosa et al sought to identify a synthetic activator of gamma delta T lymphocytes that has comparable immunostimulatory activity as natural phosphoantigens; Espinosa et al discovered BrHPP enabled immunostimulation of human gamma delta T lymphocytes (See Espinosa et al, abstract).

Espinosa et al first perform a control run using the known, natural phosphoantigen 3-formyl-1-butyl-pyrophosphate (3fbPP); peripheral blood lymphocytes were cultured at an initial concentration of 10^6 cells/mL in the presence of 10nM 3fbPP and 100 U/mL IL-2 for a 15 day period (See Espinosa et al, Pg. 18338, col. 1). Espinosa et al report significant expansion of the gamma delta T lymphocytes, including compositions comprising greater than 95% TCR Vδ2 positive cells (gamma delta T lymphocytes) (See Espinosa et al, Pg. 18338, col. 2).

Espinosa et al then perform an experimental run using several different concentrations (12.5, 25, 100 nM) of BrHPP as the activator instead of the natural 3fbPP (See Espinosa et al, Pg. 18340, col. 1-2 & Fig. 4).

Espinosa et al do not specifically describe the culture conditions of the experimental run, while they do state that peripheral blood cells were used, they are silent on the initial cell count, the length of the culture period, and whether or not IL-2 was added to the culture. However, it appears the culture conditions for the experimental run were identical to the conditions of the control run: 10^6 cells/mL were present in initial culture as well as 100 U/mL of IL-2, and the culture was maintained for 15 days. One of ordinary skill in the art would assume that for results to be comparable between the immunostimulatory activity of the 3fbPP and BrHPP, the culture conditions were identical. Therefore, in the absence of evidence to the contrary, it is assumed Espinosa et al performed a method for activation of a gamma delta T lymphocyte composition comprising culturing peripheral blood lymphocytes (PBL) in the presence of BrHPP (a synthetic activator of gamma delta T lymphocytes) and IL-2.

However, even if the culture conditions described for the control run were not duplicated in the experimental run, it would have been well within the purview of one of ordinary skill in the art to optimize the duration of the culture period, the cell density before and during culture, and the concentration of the activator compounds (BrHPP and IL-2) to those values recited in the instant claims, as each of the parameters was recognized in the art as acceptable values for T lymphocyte cell culture, or were recognized as result effective variables, which would have been routinely optimized by the artisan of ordinary skill.

With regards to the cell count of the initial cell sample, the current claims require an initial sample comprising at least 50 million mononuclear cells. While Espinosa et al disclose the concentration of the initial sample (10^6 cells/mL) they do not disclose the volume provided. However it is maintained that, in the field of cell culture, the starting cell count is generally recognized to be a result effective variables that directly affect the final cell number produced by the culture. Therefore, the sample of Espinosa et al may be 'scaled up' to involve 50-100 mL of

sample, which would contain 50-100 million cells. The scaling up of the original sample size would produce a correspondingly greater number of delta gamma T lymphocytes in the end, including over 100 million functional and viable gamma delta T cells. (claims 21 and 24)

With regards to the concentration maintained throughout the culture period, Espinosa et al reports providing the cells at a concentration of 1 x 10^6 cells/mL (See Espinosa et al, Pg 18338, col. 1, "Cell Culture"), which is less than 5 x 10^6 cells/mL. Presumably Espinosa et al maintain the cell culture at this density, though they do not explicitly state such. However, Skea et al is relied upon to show the art taught cultures of mononuclear cells for enrichment of gamma delta T lymphocytes were routinely maintained at densities of 1 x 10^5 cells/mL (See Skea et al, Pg. 526, col. 1, "T Cell Culture"). Therefore, it is submitted that one of ordinary skill in the art, at the time the invention was made, would have been able to determine an appropriate concentration at which to maintain the cell culture of Espinosa et al, the concentration being less than 5 x 10^6 cells/mL. (claims 21, 24, 29-32, 34 and 51)

With regards to the concentration of the IL-2 provided in the culture, while Belmant et al disclose using 50-100 U/mL of IL-2, the concentration of the cytokines was recognized as a result effective variable that would have been routinely optimized by the artisan of ordinary skill. In support, see Garcia et al. Garcia et al measure gamma delta T cell response to IL-2, and report that in three out of four cell clones tested, the proliferation rate of gamma delta T cells increased with increasing concentrations of IL-2 (See Garcia et al, Fig. 2). In all four cell clones tested, an increase was achieved with 10 ng/mL of IL-2 (10 ng/mL = approximately 163 U/mL, using conversion factor of 1 ng = 16.3 U). Therefore, use of IL-2 at a concentration of 163 U/mL was recognized as a concentration at which gamma delta T lymphocytes are successfully activated,

and thus optimization to such a range would have been within the purview of one of ordinary skill in the art, as the general range was taught by the art (i.e. Garcia et al). (claims 33, 53, 54)

Generally, differences in concentration will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration is critical or produces unexpected results. Where the general conditions of a claim are disclosed by the prior art it is not inventive to discover the optimum or workable ranges by routine experimentation, See *In re Aller,* 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

With regards to the concentration of the cytokine IL-2, while Espinosa et al teach the concentration of the IL-2 to be 100 U/mL (See Espinosa et al, Pg. 18338, col. 1) the concentration of the cytokines was recognized as a result effective variable that would have been routinely optimized by the artisan of ordinary skill. In support, see Garcia et al. Garcia et al measure gamma delta T cell response to IL-2, and report that in three out of four cell clones tested, the proliferation rate of gamma delta T cells increased with increasing concentrations of IL-2 (See Garcia et al, Fig. 2). In all four cell clones tested, an increase was achieved with 10 ng/mL of IL-2 (10 ng/mL = approximately 163 U/mL, using conversion factor of 1 ng = 16.3 U). Therefore, use of IL-2 at a concentration of 163 U/mL was recognized as a concentration at which gamma delta T lymphocytes are successfully activated, and thus optimization to such a range would have been within the purview of one of ordinary skill in the art, as the general range was taught by the art (i.e. Garcia et al). (claims 33, 53, 54)

It then naturally follows that because all of the above discussed variables directly effect the final gamma delta T lymphocyte cell count and concentration in the final composition, it would have been within the purview of one of ordinary skill in the art to optimize any and each of the variables so as to produce a composition with any desired cell concentration and cell count,

including at least 80% gamma delta T lymphocytes and at least 100 million cells. In the instant reference Espinosa et al report a final gamma delta T lymphocyte population which comprises approximately 63% of total lymphocytes (See Espinosa et al, Fig. 4a); alternatively, Espinosa et al teach the concentration of BrHPP directly effects the final gamma delta T lymphocyte count (See Pg. 18340, col. 2 & Fig. 4B). Thus one would have expected success optimizing the cell count and gamma delta T lymphocyte cell proportion because these variables are directly controlled by the culture steps and parameters discussed above, particularly the concentration of BrHPP; it would be well within the purview of one of ordinary skill in the art to optimize the culture conditions (adjust time between passages, adjust concentration of activation agents) in order to create a gamma delta T lymphocyte composition with the desired cell numbers and proportions (Claim 34).

Still further, regarding the cytokines used in the experiments of Espinosa et al, while Espinosa et al added IL-2 to their media, it would further have been obvious to one of ordinary skill in the art at the time the invention was made to alternatively add IL-15 to the culture medium. In support see Garcia et al; Garcia et al teach that specific cytokines, including IL-2 and IL-15 are known to enhance gamma delta T lymphocyte activation in the presence of an activator of gamma delta T cells (See Garcia et al, Pg. 4324 & Fig. 2). Garcia et al unexpectedly found IL-15 to be a more potent induces of gamma delta T cell proliferation than IL-2 (See Garcia et al, Pg. 4324, col. 2). Therefore, it would have been well within the purview of one of ordinary skill in the art to alternatively utilize IL-15 in place of, or in addition to, the IL-2 used by Espinosa et al (Claims 21 & 32). One of ordinary skill in the art would be motivated to substitute IL-15 for IL-2 in the method of Espinosa et al because Garcia et al teach IL-15 is more potent than IL-2 in enhancing gamma delta T cell proliferation. One would expect success based on the experimental findings of Garcia et al, which show successful activation and proliferation of

gamma delta T cells in the presence of IL-15 and an activator compound. Regarding the concentration of IL-15 used, Garcia et al use of up to 100 ng/mL of IL-15, but report the effect of IL-15 on gamma delta T cell proliferation was dose dependent (See Garcia et al, Pg. 4324, col. 2). Therefore, as discussed above, while applicant specifically claims a cytokine concentration in the range of 150 U/mL to 500 U/mL, manipulation and optimization within this range would be well within the purview of one of ordinary skill in the art based on the desired activation and proliferation rate of the cells as well as how often the cells are passaged (Claims 33 and 52).

Finally, while Espinosa et al teach use of peripheral blood lymphocytes, they do not specifically teach separating whole blood by cytapheresis prior to the culture method described above, nor do they teach using previous frozen samples of blood. However, at the time the invention was made it would have been well within the purview of one of ordinary skill in the art to obtain a blood sample at a first point in time, separate the whole blood sample by cytapheresis into individual components, freeze the individual components by appropriate means known in the art, and then at later time, thaw the desired platelet component for use in the method of Espinosa et al (Claims 23 and 25). In support see Valeri; Valeri teaches the basic protocols for obtaining, separating and storing blood. Valeri teaches cytapheresis allows separation of specific components of blood from whole blood (See Valeri, Pg. 1, col. 1). Separating the platelets via 'plateletpheresis' removes the majority of red blood cells and plasma components, thereby increasing the proportion of gamma delta T lymphocytes present in the initial cell sample, which is desirable in the method of Espinosa et al, as it would ensure a higher proportion of gamma delta T lymphocytes for culture and expansion, resulting in a greater end cell count. Additionally, Valeri teaches methods for frozen storage of separated platelets (See Valeri, Pg 297), which is desirable when the blood sample must be stored for a period of greater than 4 hours prior to use in the method of Espinosa et al (See Valeri, Pg. 6, col. 1). One would expect success separating the

desired platelet component via cytapheresis and storing the separated components in a frozen state for future use, because such methods and procedures are common in the art of hematology, as demonstrated by the teachings of Valeri. (claims 23 and 25)

Therefore the invention as a whole would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ALLISON M. FORD whose telephone number is (571)272-2936. The examiner can normally be reached on 8:00-6 M-Th.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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